Kidney International, Vol. 53 (1998), pp. 67–75

Serum α2-macroglobulin and α1-inhibitor 3 concentrations are increased in hypoalbuminemia by post-transcriptional mechanisms

Frazier T. Stevenson, Stephanie Greene, and George A. Kaysen

VANCHCS, Pleasant Hill, and Department of Medicine, Division of Nephrology University of California Davis, Davis, California, USA

Serum α2-macroglobulin and α1-inhibitor 3 concentrations are increased in hypoalbuminemia by post-transcriptional mechanisms. In both the nephrotic syndrome (NS) and hereditary analbuminemia in the Nagase analbuminemic rat (NAR), the plasma protein concentration is nearly normal since albumin is replaced by several high molecular weight proteins. In rats these include the protease inhibitors α2-macroglobulin (α_2M), a 720 kDa positive acute phase protein (APP) and α1-inhibitor 3 (α1-I3), a 180 kDa negative APP. There is no known stimulus to increase α1-I3 synthesis, but like albumin and other negative APPs its synthesis decreases during inflammation by transcriptional down-regulation. In hypoalbuminemic states gene transcription of other positive and negative APPs is increased. We report that α_2M was increased significantly (12-fold) in NAR and by approximately 50-fold in rats with NS compared to control. The α1-I3 concentration was twice normal in NAR or NS compared to controls, providing approximately half of the total plasma protein. Infusion of human albumin into NAR to raise albumin levels > 20 mg/ml for 24 hours caused a significant decrease in α1-I3 (24.8 ± 0.6 to 18.7 ± 0.6 mg/ml, $P < 0.0001$), equal in magnitude to that caused by 250 μg/100 g of endotoxin (23.0 ± 1.1 to 18.6 ± 0.6, $P < 0.01$). The effect of albumin was not an acute phase response since it also suppressed α_2M (239 ± 10 to 205 ± 11 μg/ml, $P < 0.005$). Turnover of ^{125}I labeled α_2M and α1-I3 was then measured in controls, NAR and in two models of the nephrotic syndrome in rats (Heymann nephritis, HN; adriamycin-induced, ADR), yielding fractional catabolic rates (FCR), which at steady state equals synthesis. The serum α_2M concentration was increased ≈50-fold and was proportional to synthesis ($r = 0.91$ $P < 0.001$). α2-Macroglobulin synthesis increased by 12-fold in NAR and 50-fold in NS. In contrast, hepatic α_2M mRNA increased only 30% in NAR and twofold in NS, suggesting post-transcriptional regulation. Fractional catabolic rates were not decreased and played no role in increasing serum α_2M in NS or NAR. The α1-I3 concentration and synthesis increased twofold from controls in both NAR and NS. However, hepatic α1-I3 mRNA was not increased in NAR and increased only 50% in NS. Unlike α_2M, serum α1-I3 correlated negatively with FCR ($-r = 0.66$, $P < 0.01$). In conclusion, both α1-I3 and α_2M concentration are increased in hypooncotic states by increased synthesis regulated post-transcriptionally, supporting plasma protein concentration when albumin is lost in urine or not synthesized.

The nephrotic syndrome is a consequence both of altered glomerular permselectivity, allowing plasma proteins of intermediate size to escape into the urine [1–3], and the alteration in synthesis and catabolism of plasma proteins [4–6], resulting in an increased plasma concentration of many proteins of high molecular weight that are too large to be lost in the urine to any significant extent [7]. We and others observed that total plasma protein concentration remained well-preserved in the nephrotic syndrome despite significant urinary loss not only of albumin, but of transferrin [8, 9], ceruloplasmin [10–12] and immunoglobulins [13]. Furthermore, plasma protein concentration, and even the relationship between plasma protein concentration and colloid osmotic pressure–oncotic pressure (π) is preserved in rats with hereditary analbuminemia, the Nagase analbuminemic rats (NAR) [14], suggesting that increased levels of other plasma proteins may play a homeostatic role in preserving plasma protein concentration or π.

We previously reported that synthesis of a group of proteins secreted specifically by the liver, including both positive (fibrinogen) and negative (albumin, transferrin, and apo A-I) acute phase proteins, was increased both in the nephrotic syndrome and in NAR [9, 15–17]. In these cases increased synthesis was regulated at the level of transcription [5, 9, 16–19]. This unusual pattern of gene expression led us to hypothesize that genes encoding a specific group of negative and positive acute phase proteins were positively regulated in hypooncotic states, a pattern distinct from that which occurs in the presence of inflammation.

Macroglobulins are a genetically related class of protease and cytokine inhibitors [20, 21] that are produced primarily in the liver [22–24]. Their synthesis is regulated either as positive (α1- and α2-macroglobulin) [22, 25–27] or negative (α1-inhibitor 3) [28, 29] acute phase proteins. They act as modulators of the acute phase response [30] and bind to and moderate the function of cytokines [30–32], a variety of growth factors [33] and potent proteases [34, 35]. It has been observed that one of these proteins, α2-macroglobulin (α_2M) is also increased in the nephrotic syndrome in patients [36].

In the rat the predominant macroglobulin is α1-inhibitor 3 (α1-I3), a monomeric (180 kDa) negative acute phase protein similar in structure to each of the four α_2M subunits. α2-Macroglobulin is a 720 kDa tetrameric vigorously responding positive acute phase protein in the rat [26]. In humans with the nephrotic syndrome, total plasma protein concentration is also maintained by increased levels of high molecular weight globulins, mainly α_2M [36, 37]. As in humans, the level of α_2M is elevated in

Key words: nephrotic syndrome, protein synthesis, fractional catabolic rate, analbuminemia, protease inhibitors.

Received for publication July 2, 1997
and in revised form August 14, 1997
Accepted for publication August 15, 1997

nephrotic rats [38]. There is no known stimulus for synthesis of the negative acute phase protein α1-I3 [28, 29]. The metabolism of this protein in hypooncotic states is unknown. Our hypothesis, if it can be extended to these protease inhibitors, would suggest that synthesis of both α_2M and α1-I3 should be stimulated at the transcriptional level in both the nephrotic syndrome and NAR.

In these experiments, we sought to establish the basal levels of these macroglobulins in rats with either nephrotic syndrome [Heymann nephritis (HN) or adriamycin-induced nephrotic syndrome (ADR)] or NAR, and to investigate the mechanism of changes in the serum levels of these proteins.

METHODS

Reagents

α2-Macroglobulin and α1-I3 were purified from the plasma (ethylene diamine tetraacetic acid; Fisher Chemical Co., Fair Lawn, NJ, USA) of 12 rats which had been injected subcutaneously with 0.5 c.c. of turpentine 48 to 72 hours prior to bleeding. The chilled plasma was adjusted to 5% PEG (carbowax-PEG 8000; Fisher) by the addition of 50% PEG solution and stirred on ice for 30 minutes. All steps were performed at 4°C. The solution was centrifuged at 2500 rpm for 15 minutes; the supernatant was saved and adjusted to 13% PEG, and stirred and centrifuged as before. The pellet was dissolved in 50 mM Tris (Trizma Base; Sigma Chemical Co., St. Louis, MO, USA), pH 7.5, 50 mM NaCl to 1/2 the original plasma volume.

The protein solution was applied to an affi-gel Blue column (2.5 cm $\times$ 13 cm; Bio Rad, Richmond, CA, USA) equilibrated in the same buffer, the flow through was collected and adjusted to pH 8.0 with 1 N NaOH and applied to a DE-52 (Whatman Chemical Co., Maidstone, UK) column (2.5 cm $\times$ 20 cm) equilibrated in 50 mM Tris pH 8.0, 50 mM NaCl (Fischer), 0.02% NaN_3 (Fischer). After washing, elution of the two proteins was accomplished with a gradient in 50 mM Tris, pH 8.0, from 50 mM NaCl to 250 mM NaCl (total volume = 500 ml). Those fractions that eluted at the front of the rather broad peak contained α_2M, while later fractions contained a mixture of α_1M and α1-I3. Sodium dodecyl sulfate—polyacrylamide gel electrophoresis (SDS-PAGE) [39], on both unreduced and reduced samples, was used to assess which fractions to pool for the two proteins. Two separate pools were made, concentrated using a Filtron centrifugal concentrator (MWCO = 50 kDa), and chromatographed separately on Sephacryl S300 (2.5 cm $\times$ 80 cm; Pharmacia Fine Chemicals, Uppsala, Sweden). Purity was assessed by SDS-PAGE.

Polyclonal antibodies were obtained for each purified protein by subcutaneous injection into New Zealand White rabbits by first using complete Freund's adjuvant followed by incomplete adjuvant. Specificity for each antibody was established first by determining that only a single band of immunoprecipitation occurred using the Ochterlony double diffusion technique and then by Western blot [39]. Electroimunodiffusion assays were then developed for each protein that were both linear in the concentration range of interest and that exhibited parallelism with diluted controls. Care was taken not to repeatedly freeze thaw standards.

Experimental models

With the exception of NAR all rats were male Sprague Dawley rats purchased from Bantin Kingman Farms (Fremont, CA, USA). All studies were performed in males.

Passive Heymann nephritis. Sprague-Dawley rats weighing 120 to 150 g were injected intraperitoneally with sheep FX1A antiserum (Dr. Florence Hutchison, Charleston, SC, USA). Proteinuria begins at nine days and reaches a plateau at 11 days in this model [4]. All studies were performed after 11 days.

Adriamycin-induced nephrotic syndrome was established by injecting 5 mg/kg of adriamycin into a tail vein approximately 21 days prior to the study [40].

Nagase analbuminemic rats (NAR) were raised in our own colony, originally derived from a colony of animals donated to us by Dr. Jaap Joles (Utrecht, the Netherlands). They were derived by Cesarean section and care was taken to assure that they remain pathogen free. Every four generations NAR males are bred with normal Sprague-Dawley female rats and the subsequent f1 generation is back bred with NAR males. The resulting f2 generation is subsequently tested for the presence of plasma albumin using immunochemical techniques.

Since adriamycin is immunosuppressive and might alter the acute phase response, we performed our initial studies in nephrotic animals in the HN model. The antibody that creates HN is directed to a receptor that is closely related to the α_2M receptor, the lipoprotein-like receptor protein (LRP) [41]. When we found that α_2M catabolism was increased in HN (to be presented below), we extended our study to the ADR model to assure that our results were not model dependent.

Determination of α2-macroglobulin and α1-inhibitor 3 levels in hypooncotic states and after lipoprotein-like receptor protein administration

The basal levels of α_2M and α1-I3 were measured in 24 normal control animals (C; Sprague-Dawley rats; N = 24), 24 Nagase analbuminemic rats (NAR) and 27 rats with passive Heymann nephritis (HN). Passive Heymann nephritis was induced by intraperitoneal injection of sheep anti-FX IA antiserum as described previously [4]. Proteins were measured by immunoelectrophoresis as previously described [42].

Graded doses of endotoxin [lipopolysaccharide (LPS)] were then administered as a bolus injection of 0.01, 0.15, 2.0, 25 or 250 μg/l00 g body wt into a tail vein. All injections were in 0.5 cc volume. Saline was administered as the control. Animals were fasted for 24 hours following injection since animals receiving the two higher doses of LPS did not eat, and we wanted to assure that differences in dietary intake played no role in the outcome. Blood was obtained from a tail vein at 24 hours. Six animals in each group were injected with each dose of LPS. The concentration of α1-I3 was determined in the initial and final (24 hr) serum samples in all groups of animals.

Determination of the effect of infusion of hyperoncotic human albumin on the plasma levels of α2-macroglobulin and α1-inhibitor 3

Maintaining plasma albumin concentration near the normal range in nephrotic rats requires continuous infusion through a catheter in the jugular vein tunneled subcutaneously [43]. In preliminary studies during which saline was infused continuously into NAR using subcutaneously tunneled catheters we found an increase in the plasma concentration of acute phase proteins even when meticulous care was paid to sterility of technique. No such changes occurred when we administered either albumin or saline

by intermittent bolus injections, since no surgery was required. Thus, we chose to infuse albumin into NAR rather than add the additional trauma of surgery to the model. We used NAR since no urinary protein losses occur and, therefore, continuous infusion is unnecessary to elevate plasma albumin concentration or π. Hyperoncotic human serum albumin (25%) (HSA) was injected intravenously into 20 NAR to establish the effect on the plasma concentration of α_2M and α1-I3. Human serum albumin was administered in three boluses into a tail vein using a #23 scalp vein. The first bolus consisted of 400 mg/100 g body wt of human albumin (to provide a normal total albumin mass) and the following two of 200 mg/100 g body wt each. Boluses were administered in 12-hour intervals. Albumin concentration was maintained above 20 mg/ml. Serum was obtained for measurement of α_2M and α1-I3 initially and immediately prior to the third dose of albumin in order to establish the effect of albumin infusion on the plasma levels of these three proteins. Immediately following administration of the third dose of albumin, LPS was administered in graded doses to establish the effect of albumin infusion on the response to LPS administration. Albumin was infused in these animals 12 hours after LPS administration to assure that albumin levels remained $>$ 20 mg/ml throughout the experimental period.

Quantitation of mRNA by dot blot hybridization

To obtain livers, rats were then anesthetized with sodium pentobarbital (30 mg/kg) by intraperitoneal administration. The abdomen was incised and the liver perfused retrograde with iced saline. The liver was then removed, weighed and RNA was extracted as described subsequently. Rat liver total RNA was then isolated by homogenizing approximately 200 mg of tissue in guanidinium isothiocyanate/Na acetate/mercaptoethanol using the method of Srivastava, Srivastava and Schonfeld [44]. The quality of the RNA isolated was assessed by visualization following acridine orange staining on 1.5% agarose/formaldehyde gels, as well as by Northern blotting using a probe for the constituitively expressed GAPDH gene. Any samples showing significant degradation were discarded. Probes for α1-I3, α_2M, and GAPDH were obtained from ATCC. Northern blots of 10 μg/lane of RNA were initially run with each probe to determine hybridization conditions and probe specificity; single bands of the expected molecular weights were obtained prior to dot blotting. ^{32}P-dCTP labeled probes were made by random hexamer extension (RadPrime kit, BRL). Ten microgram samples of each RNA were applied to a dot blot manifold, fixed by NaOH, and hybridized overnight at 65°C with the appropriate probe. Blots were washed in 1 to 2% sodium dodecyl sulfate/0.05 to 0.1 $\times$ SSC at 65°C for one hour, prior to developing for 2 to 16 hours on a Phospho-Imager plate. Dot intensity was quantified by NIH Image analysis of the scanned images. mRNA quantification is expressed as a ratio of the dot intensity of specific genes under study to GAPDH, and expressed ratios are the means of three to six measurements.

Rates of turnover of α2-macroglobulin and α1-I3 in nephrotic and analbuminemic rats

α2-Macroglobulin was iodinated using chloramine T (Sigma) at a molar ratio of 0.8 moles of 125 iodine (New England Nuclear) to 1 mole of α_2M. We then determined both kinetically and electrophoretically that the iodinated macroglobulin was a mixture of native and protease activated macroglobulin. Approximately 2×10^8 dpm of the iodinated protein was then injected into a rat to remove the protease activated α_2M, which has a biological half life of approximately two to three minutes [45]. The rat was anesthetized by intravenous administration of sodium pentobarbital (20 mg/kg) immediately following injection of the iodinated α_2M. The abdomen was opened and the rat exsanguinated 15 minutes after administration of the iodinated protein. Approximately 10 cc of blood was allowed to coagulate on ice, and was then centrifuged and sterilized by millipore ultrafiltration and used within three days. The serum was finally used as the labeled protein.

Measurement of the α_2M catabolic rate was performed by intravenous injection into a tail vein (without anesthesia) of between 10^6 and 2×10^6 dpm of serum derived as above. Blood (50 μl) was obtained by nicking the tail at three minutes, 10 minutes, 30 minutes, 60 minutes, one hour, three hours, 10, 24, 48, 72 and up to 96 hours following injection or when residual counts in plasma were $\leq$ 5% of initial counts, whichever came first. Metabolic clearance was determined as described previously for other proteins [46], although the sum of exponentials was used rather than the trapezoidal method for integration with a commercially available curve fitting program (SAAM II; SAAM institute, University of Washington, WA, USA). The product of clearance, which was derived in volume per unit of time, and plasma concentration of a given protein yielded the total quantity of that protein removed per unit time. At steady state, the removal rate (catabolism plus external loss) was the average rate of synthesis over that time period [4].

Radioiodinated α1-I3 was prepared and biologically prescreened in the same way as was radioiodinated α_2M. All radiolabel was isolated to a single protein migrating at 180 kDa, and was assured both by Western blot using specific antiserum and phosphoimaging of the labeled material. The iodinated α1-I3 was then used for kinetic measurements. Urine was collected during the kinetic measurement of α1-I3 clearance in nephrotic rats, since we had previously established that substantial amounts of this protein were lost in the urine (as much as 30% of total urinary protein). Fractional catabolic rate (FCR) was the fraction of the plasma pool catabolized per unit of time. Urinary losses of α1-I3 were measured in nephrotic rats when the turnover of this protein was studied, and these losses were subtracted from total turnover in order to establish the fractional catabolic rate of α1-I3 in nephrotic animals.

Statistical methods

Data were first analyzed for normality of distribution using the Kolmogorov-Smirnov test for normality (using Sigma Stat, a commercial statistics program; Jandel Corp., San Rafael CA, USA). If data were non-normally distributed they were analyzed by the Kruskal-Wallis one way analysis of variance on ranks. If differences were detected multiple groups were then analyzed using Dunn's method for multiple comparisons. The Mann-Whitney sum test was used to make comparisons when only two groups were analyzed. Differences were considered statistically significant at $P < 0.05$. Regression analysis was performed using the methods of least squares. Results of normally distributed data are presented as mean $\pm$ SEM.

RESULTS

Macroglobulin levels, synthesis, and catabolism in hypoalbuminemic states

Basal levels of α_2M were significantly greater in HN (2284 ± 217 μg/ml) than in NAR (291 ± 13, $P < 0.001$) and were lowest in C (84 ± 4.4, $P < 0.001$ compared to both HN and NAR) prior to administration of either LPS or albumin. Basal α1-I3 was also increased significantly in both NAR (22.7 ± 0.4 mg/ml) and HN (21.2 ± 0.7 mg/ml) compared to C (10.7 ± 0.5 mg/ml). The concentration of α1-I3 in HN was about half of that of albumin in normal animals and made up the bulk of plasma protein mass. The increase in α1-I3 was surprising considering that it is normally either constituitively expressed or down-regulated by inflammation [26–29].

Effect of lipopolysaccharide administration

One mechanism that could be responsible for increased α_2M levels and that of other acute phase proteins could be an alteration in the sensitivity of hypoalbuminemic organisms to LPS. This lipophilic substance could potentially be bound to albumin and thus hypoalbuminemia could potentially augment the availability of this compound to its target cells. If this were correct, the amount of LPS necessary to cause a half maximal response should increase following replacement of albumin to the animal. For this reason we tested a dose response relationship between changed levels of α_2M following the administration of graded doses LPS as well as the effect of albumin infusion on this response in NAR. It was also not an effect of plasma pool dilution, since the concentration of another unrelated acute phase protein, α1 acid glycoprotein, did not change (data not shown).

Lipopolysaccharide caused a dose related increase in plasma α_2M in C, NAR, and HN animals with a half maximal response between 0.15 and 2 μg LPS/l00 g body wt, independent of any group (Fig. 1). Thus, the sensitivity to LPS was the same in all groups. The absolute final level of α_2M and net change in plasma α_2M level was in large part a consequence of the initial level. The absolute final level of α_2M was greatest in HN, while the percent increase (19-fold) was greatest in C and intermediate in NAR (11-fold), and least in HN (2.6-fold). Infusion of LPS into NAR previously treated with albumin led to the same final α_2M concentration as in C.

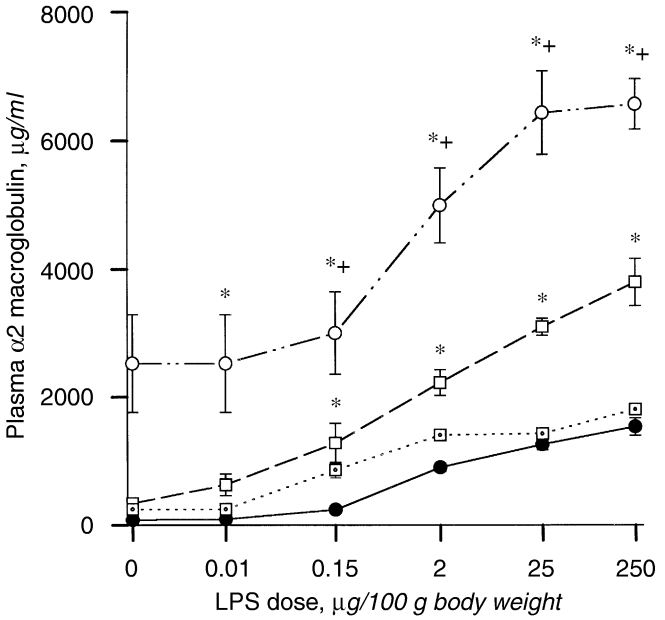

Fig. 1. Increase in plasma α2-macroglobulin level 24 hours after administration of graded doses of lipopolysaccharide (LPS) into a tail vein. Six rats (all male) were injected with each dose of LPS (0.01 μg/100 g body weight, 2 μg/100 g body weight, 25 μg/100 g body weight and 250 μg/100 g body weight) or saline. All animals were fasted for the following 24 hours but allowed free access to water. Abbreviations are: HN (○), Heymann's nephritis; NAR (□), rats with hereditary analbuminemia; control (●), normal Sprague-Dawley rats; NAR + Alb (⊡), NAR rats that received i.v. albumin infused for 24 hours prior to and following the administration of LPS to maintain a serum albumin concentration > 20 mg/ml. $^*P < 0.05$ vs. control; $^\dagger P < 0.05$ vs. NAR. Data are mean ± SEM.

Effect of albumin infusion

Albumin infusion significantly reduced both α1-I3 and α_2M levels in NAR (Fig. 2). The decrease in α1-I3 concentration was the same as that produced by LPS administration but was not a result of a generalized acute phase response, since α_2M levels also decreased significantly following albumin infusion (Fig. 2), but rose markedly after LPS treatment (Fig. 1). Hence, correction of hypoalbuminemia attenuated the elevations of both macroglobulins in NAR.

Gene expression

Surprisingly, although the concentration of α_2M was increased by 27-fold in HN compared to C, the increase in the concentration of α_2M mRNA in liver was quite small (approximately 2-fold; Fig. 3). Administration of LPS produced a robust increase in α_2M RNA in liver, demonstrating that the expected response to a stimulus known to increase transcription of this protein [22, 23, 26] was maintained. Increased levels of α_2M in hypoalbuminemic states must then be a consequence of either increased efficiency of translation of the protein from previously extant hepatic message or altered hepatic protein processing/secretion or a decrease in the fractional catabolic rate (FCR) of the protein.

Rats with HN had an approximate 50% increase in α1-I3 mRNA (Fig. 4), inadequate to entirely explain the doubling in serum concentration. The mRNA levels in NAR rats did not differ significantly from control, yet serum concentration was also increased by approximately twofold. In all groups, α1-I3 mRNA levels decreased following LPS administration, as would be expected in this negative acute phase reactant (Fig. 4). The doubled serum concentration of α1-I3 in NAR and nephrotic animals is at least in part a consequence of post-transcriptional events, as in α_2M. Post-transcriptional events that might alter the concentration of a protein in plasma include changes in the protein fractional catabolic rate.

Turnover of α2-macroglobulin and α1-inhibitor 3 in NAR and nephrotic rats

To determine whether changes in synthesis or in FCR were responsible for increasing levels of α_2M and α1-I3, we measured the turnover of the iodinated proteins at steady state. These measurements will yield both a FCR and a synthesis rate at steady state [4, 6, 46]. Initially we determined that α_2M FCR was increased significantly in HN, an unanticipated finding (Table 1). Since HN is initiated by an antibody to GP330, a protein with

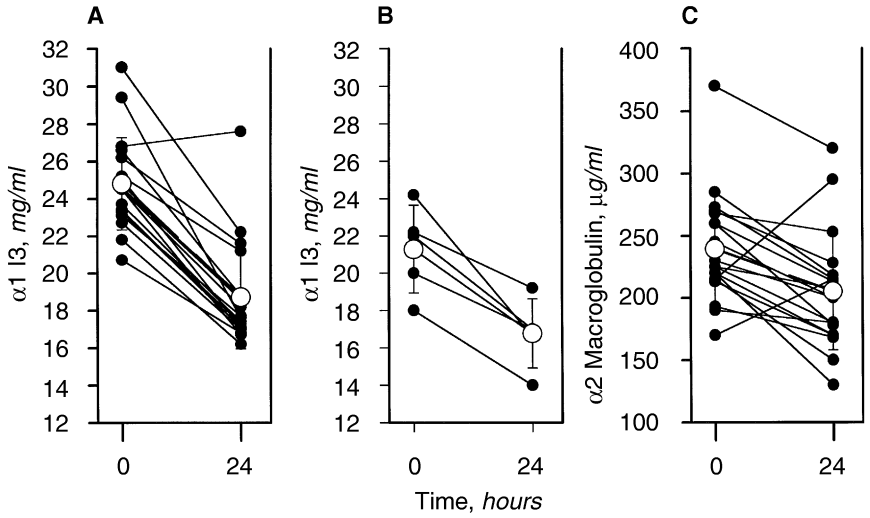

Fig. 2. Effect of infusion of albumin or lipopolysaccharide on plasma α1-inhibitor 3 (α1-I3) concentration and of albumin on plasma α2-macroglobulin (α_2M) concentration in rats with hereditary albuminemia (NAR). Blood was drawn from a tail vein prior to infusion and then at 24 hours. Hyperoncotic (25%) human serum albumin was infused first as a bolus of 400 mg/100 g body wt and then a second dose of 200 mg/100 g body wt was administered 12 hours later. No albumin was administered to the rats receiving LPS 250 μg/100 g body wt.

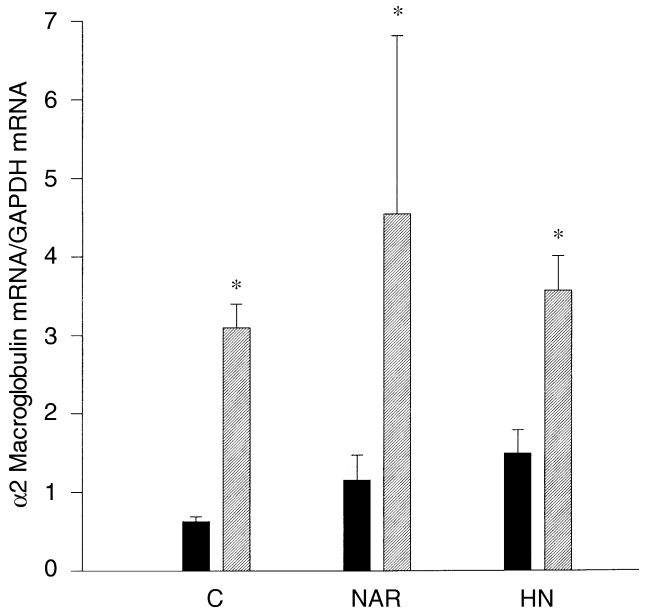

Fig. 3. Hepatocyte mRNA levels of α2-macroglobulin (α_2M) in control and hypooncotic states. Total RNA from the experimental groups was dot blotted and analyzed with radioactive probes for α_2M and for the constituitive control gene GAPDH. Data are expressed as mean α_2M/GAPDH ratio (means ± SEM from at least 3 experiments). (■ saline-injected; ▨ LPS-injected; $^*P < 0.05$ vs. no LPS.)

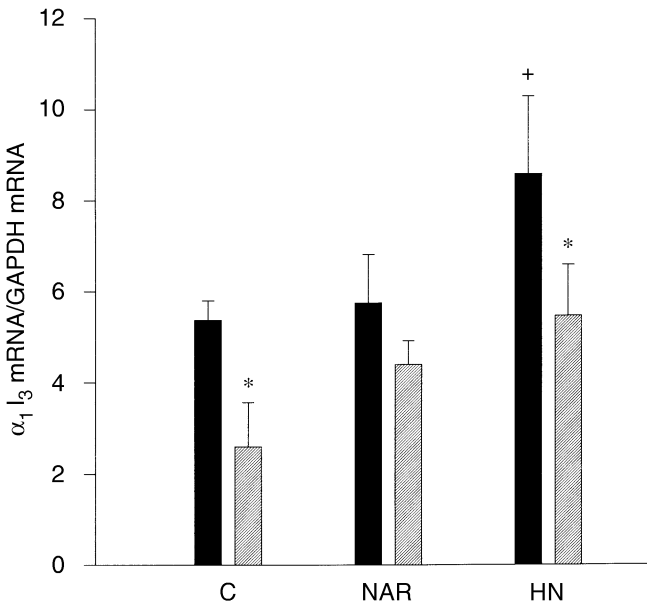

Fig. 4. Hepatocyte mRNA levels of α1-inhibitor 3 (α1-I3) in control and hypooncotic states. Total RNA from the experimental groups was dot blotted and analyzed with radioactive probes for α1-I3 and for the constituitive control gene GAPDH. Data are expressed as a mean α1-I3/GAPDH ratio (means ± SEM from at least 3 experiments). (■ saline-injected; ▨ LPS-injected; $^*P < 0.05$ vs. no LPS; $^+P < 0.05$ vs. control.)

close homology to the α_2M receptor [41], we were concerned that the HN model might produce artifactual results when measuring macroglobulin catabolism. For this reason we performed the rest of our catabolic studies using the adriamycin model.

α2-Macroglobulin was increased approximately 20-fold in NAR and 40-fold in HN and 50-fold in ADR (Table 1). The elevated level of α_2M was entirely a consequence of increased synthesis (Table 1). The synthetic rate was directly and linearly proportional to plasma concentration (Fig. 5). In contrast, α_2M FCR was independent of plasma concentration and not different in controls, NAR, or ADR, but was significantly increased in HN (Table 1), a change that should have favored a decreased plasma concentration of this protein in this group of animals. Thus, a reduction in FCR played no role in supporting an increased plasma concentration of this protein. α2-Macroglobulin is sufficiently large (720 kDa) so that it is not lost in the urine in significant amounts even in nephrotic animals (data not shown).

The mechanism for the unexpected elevations in α1-I3 plasma concentrations was more complex. The plasma concentration of α1-I3 was significantly greater in ADR than in NAR despite significant urinary losses in nephrosis (Table 2). Urinary losses of α1-I3 were about 20% that of albumin, representing a large fraction of non-albumin protein in the urine of nephrotic rats. The

Table 1. α2-Macroglobulin metabolism in normal analbuminemic and nephrotic rats

Group	Serum concentration of α2 macroglobulin *mg/ml*	Synthesis *mg/100 g body wt/hr*	FCR *% plasma pool/hr*
Control $N = 8$	0.0868 ± 0.0275	0.0443 ± 0.0175	9.16 ± 1.125
NAR $N = 8$	1.56 ± 0.226[a]	0.531 ± 0.111[a]	9.29 ± 1.127
Adriamycin $N = 6$	3.88 ± 0.31[ab]	2.261 ± 0.341[ab]	10.93 ± 1.63
Heymann nephritis $N = 3$	2.07 ± 0.90[a]	1.58 ± 0.703[ab]	19.34 ± 1.344[abc]

Data are means ± SEM.
[a] $P < 0.05$ vs. Control
[b] $P < 0.05$ vs. NAR
[c] $P < 0.05$ vs. Adriamycin

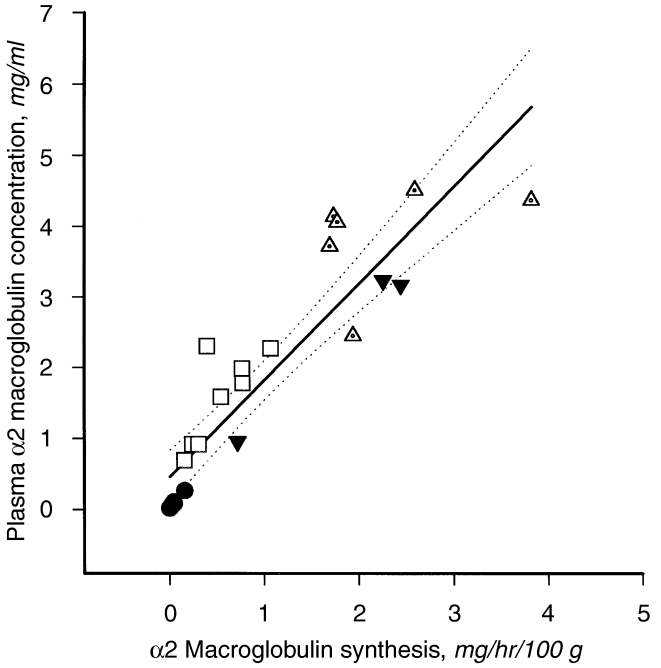

Fig. 5. Relationship between plasma α2-macroglobulin (α_2M) concentration and its rate of synthesis measured as the product of clearance of ^{125}[I]labeled native α_2M and its plasma concentration. Symbols are: (●) control; (□) NAR; (▼) HN; (△) ADR. The solid line denotes the least squares regression, and dotted lines are the 95% confidence limit. (r = 0.903; $P < 0.001$)

rate of synthesis of α1-I3 was increased twofold in both NAR and ADR and was proportional to serum concentration (Fig. 6). In contrast, FCR of α1-I3 was significantly decreased in ADR, in sharp contrast to what occurs for other proteins lost into the urine (transferrin and albumin), where the fractional catabolic rate sharply increases [4, 8, 47, 48]. α1-Inhibitor 3 FCR tended to also decrease in NAR, but this did not achieve statistical significance (Table 2). For all groups there was an inverse relationship between α1-I3 concentration and its FCR ($-r = 0.66$, $P < 0.01$). In contrast to α_2M the mechanism of increased plasma concentration of α1-I3 was multifactorial, with both increased synthesis and decreased FCR contributing to its increased plasma levels.

DISCUSSION

In both the nephrotic syndrome and in hereditary analbuminemia the concentrations of many liver derived proteins are increased in plasma [14]. In the situation of NAR, where no urinary protein losses occurs, plasma protein concentration is essentially the same as in non-nephrotic animals [49], and the relationship between plasma protein concentration and π is also nearly normal [14], suggesting that a regulatory mechanism(s) may defend plasma protein concentration or π when the albumin concentration is reduced either as a consequence of lack of synthesis or external losses. The increased synthesis of proteins that replace albumin takes place primarily or exclusively in the liver. Furthermore, transcription of a group of liver derived proteins (albumin [5, 18, 19], transferrin [9], apo A-I [16], and fibrinogen [17]) is increased, suggesting that transcriptional upregulation of a group of proteins might be regulated in a manner analogous to what occurs in the case of the acute phase response.

Marsh and Sparks suggested that the hyperlipidemia of the nephrotic syndrome was, at least in part, a consequence of a generalized hepatic response [50]. However, they found that not all liver derived proteins behaved in the same way. The increase in synthesis of apo B and apo E was not as great as was the increase in apo A-I synthesis, while apo C synthesis was not increased at all [51].

While small amounts of α_2M are synthesized in extrahepatic tissue [24, 52], it is unlikely that these tissues contribute importantly to increased plasma concentrations of this protein. α1-Inhibitor 3 is only liver derived [53]. An increase in plasma concentration of either protein then must reflect either an increase in hepatic protein synthesis or a decrease in the FCR for the specific protein or both (as occurs for apo A-I) [6]. Since Vaziri noted that α_2M concentration was increased in nephrotic patients, we speculated that increased α_2M would be a consequence of increased synthesis and that this regulation would transcriptional, as in the case of another class II acute phase protein fibrinogen [17]. Furthermore, since transcription of albumin, apo A-I, and transferrin were increased, we speculated that that of α1-I3 would also be regulated similarly. α1-Inhibitor 3 is smaller than α_2M and is thus capable of contributing more to π. It is also present in relatively high concentrations in rodents [28, 29] and hence could play a significant role in maintaining plasma protein content. We have now established that synthesis of both of these protease inhibitors is indeed increased in hypoalbuminemic states, and in the case of α_2M synthesis rates are increased massively. Plasma α_2M concentration in nephrotic rats not exposed to LPS is greater than in normal animals given a dose of LPS that induces a maximal increase in plasma α_2M levels. Thus, hypoalbuminemia is a more effective means of increasing plasma α_2M than is the acute phase response, normally considered to be the primary stimulus for synthesis of this protein. The response to hypoalbuminemia is not a consequence of increased sensitivity of animals with low albumin levels to LPS since the dose response curve is not shifted. Furthermore, the mechanisms for increased synthesis resulting from hypoalbuminemia and from the acute phase response are different. While LPS administration induces a robust increase in α_2M mRNA in the livers of both nephrotic and normal rats, hepatic α_2M mRNA levels are increased only slightly

Table 2. α1-Inhibitor 3 (α1-I3) metabolism in normal analbuminemic and adriamycin-induced nephrotic rats

Group	Serum α1-I3	Urinary α1-I3	Urinary albumin	α1-I3 Synthesis	FCR % *plasma pool/hr*
	mg/ml			*mg/100 g body wt/hr*	
Control $N = 5$	16.4 ± 0.30	ND	ND	5.41 ± 0.82	8.30 ± 0.85
NAR $N = 5$	29.2 ± 1.7[a]	ND	ND	9.27 ± 1.0[a]	6.13 ± 0.47
Adriamycin $N = 5$	36.4 ± 3.1[a]	48.5 ± 10.2	279 ± 47	10.26 ± 1.1[a]	5.84 ± 0.63[a]

Means ± SEM. ND is not done.
[a] Difference from Control ($P < 0.05$)

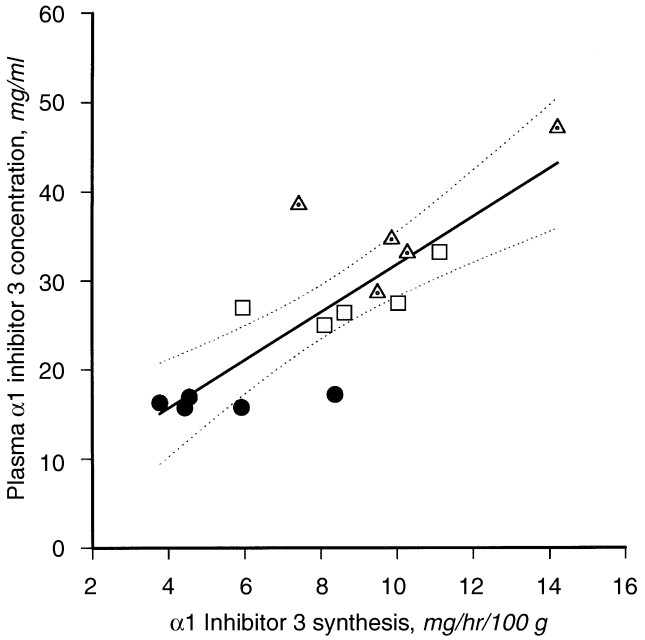

Fig. 6. Relationship between plasma α1-inhibitor 3 (α1-I3) concentration and its rate of synthesis measured as the product of clearance of ^{125}[I]labeled native α1-I3 and its plasma concentration. Symbols are: (●) control; (□) NAR; (▼) HN; (◬) ADR. The solid line denotes the least squares regression, and dotted lines are the 95% confidence limit. (r = 0.817; $P < 0.001$)

in the nephrotic syndrome or NAR, yet α_2M synthesis is increased by ≈50-fold.

The biology of α1-I3 was unexpected. The rate of synthesis of α1-I3 was quite high, similar to that reported for albumin in normal animals [4, 5]. α1-Inhibitor 3 is small enough for substantial amounts to be lost in the urine of nephrotic rats, and we find its urinary loss to be about 20% that of albumin. Despite these urinary losses, plasma concentration increased to levels comparable to that of albumin in non-nephrotic animals. This increase in α1-I3 plasma level was a consequence both of increased synthesis and decreased FCR. This protein is a negative acute phase protein, and as far as we are aware there is no known stimulus to increase either its levels or its rate of synthesis save the conditions described here: the nephrotic syndrome and analbuminemia. The observation that plasma α1-I3 levels decrease following administration of albumin and that synthesis is increased both in analbuminemic and nephrotic rats suggests that the rate of synthesis of this protein is in part regulated by either albumin concentration or π. Like α_2M, this regulation is at least in part post-transcriptional, suggesting that reduced albumin concentration or π either directly or indirectly alters hepatic protein processing or the efficiency of translation of some proteins from existent mRNA in liver.

The nephrotic syndrome results in changes not only in synthesis of plasma proteins, but also in their FCRs. Fractional catabolic rate for a protein is a function of the catabolic rate constant. An FCR that remains constant despite changes in the plasma concentration of a given protein is a consequence of unchanging first order kinetics that are neither saturated nor regulated. Albumin is a protein whose plasma concentration is in part regulated or supported by alterations in its FCR. When albumin is reduced as a consequence of reduced synthesis, FCR decreases serving to mitigate the decrease in albumin concentration that would otherwise result from a decrease in synthesis [54]. In contrast, when albumin levels are increased as a result of albumin infusion FCR increases and returns albumin levels back toward normal. In the nephrotic syndrome this positive relationship between albumin concentration and its FCR is inverted [48, 55, 56], leading to a paradoxical increasing FCR when serum albumin is reduced. This is also true for transferrin, another protein lost extensively in the urine in the nephrotic syndrome [8, 9]. This altered relationship between the serum concentration of these intermediate sized proteins and their FCRs in the nephrotic syndrome has been postulated to result from their increased renal catabolism. It was postulated that a much larger quantity of these proteins was filtered than could be found in urine, but that a large quantity was reabsorbed by renal tubular cells and catabolized there [47, 57, 58]. That hypothesis has not been proven, and indeed there are data suggesting that nearly all of the filtered albumin finds its way into urine [59–63]. Nevertheless, the FCRs of proteins that are lost extensively in the urine in the nephrotic syndrome are generally increased [4, 8, 55, 58, 59, 64], with the exception now of α1-I3. It is not known whether the decreased α1-I3 FCR is a regulated process or whether the catabolic site that normally processes this protein is saturated or nearly so at physiological α1-I3 concentrations. Nevertheless, a reduced FCR for this protein lost in urine is both novel and contributes to increasing its concentration in plasma. In contrast to α1-I3, α_2M FCR is independent of plasma concentration with the exception of HN. We believe that the increased FCR in HN may be model dependent due to the interaction between macroglobulins and the LRP, but clearly an increase in FCR would favor a reduced, not an increased α_2M concentration.

The effect of alterations in albumin concentration or π on

hepatocyte protein synthesis and secretion is complex, and that increased synthesis of these hepatic proteins serves a role to support plasma protein concentration at or near the normal range despite either the absence of effective albumin synthesis or the urinary losses of the proteins that normally make up the bulk of plasma protein mass. Were it not for these complex responses by the liver both hereditary analbuminia and the nephrotic syndrome would have a much greater impact on total plasma protein levels. The binding function of these macroglobulins for cytokines, growth factors, proteases, and metals may also play a role in the pathophysiology of these syndromes.

ACKNOWLEDGMENTS

The Research Service of the Department of Veterans Affairs, and technical assistance of Elaine Hoye, Hardin Jones, Jr. and Nouradine Azouzou are appreciated.

Reprint requests to George A. Kaysen, M.D., Ph.D., Division of Nephrology, TB 136, UC Davis, Davis, California 95616, USA.
E-mail: gakaysen@ucdavis.edu

REFERENCES

1. Deen WM, Bridges CR, Brenner BM, Myers BD: Heteroporous model of glomerular size selectivity: Application to normal and nephrotic humans. *Am J Physiol* 249:F374–F389, 1985
2. Gitlin D, Janeway CA, Farr LE: Studies on the metabolism of plasma proteins in nephrotic syndrome. I. Albumin, gamma-globulin and iron-binding globulin. *J Clin Invest* 35:44–55, 1956
3. Baldamus CA, Galaske R, Eisenbach GM, Krause HP, Stolte H: Glomerular protein filtration in normal and nephrotic rats: A micropuncture study. *Contr Nephrol* 1:37–49, 1975
4. Kaysen GA, Kirkpatrick WG, Couser WG Albumin homeostasis in the nephrotic rat: Nutritional considerations. *Am J Physiol* 247:F192–F202, 1984
5. Kaysen GA, Jones H Jr, Martin V, Hutchison FN: A low-protein diet restricts albumin synthesis in nephrotic rats. *J Clin Invest* 83:1623–1629, 1989
6. Kaysen GA, Hoye E, Jones H Jr: Apolipoprotein AI levels are increased in part as a consequence of reduced catabolism in nephrotic rats. *Am J Physiol* 268:F532–F540, 1995
7. Girot R, Jaubert F, Leon M, Bellon B, Aiach M, Josso F, Lepelletier O, Beguin S, Monnet JP: Albumin, fibrinogen prothrombin, and antithrombin III variations in blood, urines and liver in rat nephrotic syndrome (Heymann nephritis). *Thromb Haemostas* 49:13–17, 1983
8. Jensen H, Bro-Jorgensen K, Jarnum S, Olesen H, Yssing M: Transferrin metabolism in the nephrotic syndrome and in protein-losing gastroenteropathy. *Scand J Clin Lab Invest* 21:293–304, 1968
9. Kaysen GA, Sun X, Jones H Jr, Martin VI, Joles JA, Tsukamoto H, Couser WG, Al-Bander H: Non-iron mediated alteration in hepatic transferrin gene expression in the nephrotic rat. *Kidney Int* 47:1068–1077, 1995
10. Pedraza-Chaverri J, Torres-Rodriguez GA, Cruz C, Mainero A, Tapia E, Ibarra-Rubio ME, Silencio JL: Copper and zinc metabolism in aminonucleoside-induced nephrotic syndrome. *Nephron* 66:87–92, 1994
11. Stec J, Podracka L, Pavkovcekova O, Kollar J: Zinc and copper metabolism in nephrotic syndrome. *Nephron* 56:186–187, 1990
12. Cartwright GE, Gubler CJ, Wintrobe MM: Studies on copper metabolism: Copper and iron metabolism in the nephrotic syndrome. *J Clin Invest* 33:685–698, 1954
13. Al-Bander HA, Martin VI, Kaysen GA: Plasma IgG pool is not defended from urinary loss in nephrotic syndrome. *Am J Physiol* 262:F333–F337, 1992
14. Kaysen GA: Plasma composition in the nephrotic syndrome. *Am J Nephrol* 13:347–359, 1993
15. Kaysen GA, Hoye E, Jones H Jr, van Tol A, Joles JA: Effect of oncotic pressure on apolipoprotein A-I metabolism in the rat. *Am J Kidney Dis* 26:178–186, 1995
16. Sun X, Jones H Jr, Joles JA, van Tol A, Kaysen GA: Apolipoprotein gene expression in analbuminemic rats and in rats with Heymann nephritis. *Am J Physiol* 262:F755–F761, 1992
17. Sun X, Martin V, Weiss RH, Kaysen GA: Selective transcriptional augmentation of hepatic gene expression in the rat with Heymann nephritis. *Am J Physiol* 264:F441–F447, 1993
18. Yamauchi A, Imai E, Noguchi T, Tanaka T, Yamamoto S, Mikami H, Fukuhara Y, Fujii M, Orita Y, Kamada T: Albumin gene transcription is enhanced in liver of nephrotic rats. *Am J Physiol* 254:E676–E679, 1988
19. Kaysen GA, Jones H Jr, Hutchison FN: High protein diets stimulate albumin synthesis in nephrotic rats at the site of albumin mRNA transcription. *Kidney Int* 27:S168–S172, 1989
20. Overbergh L, Hilliker C, Lorent K, Van Leuven F, Van den Berghe H: Identification of four genes coding for isoforms of murinoglobulin, the monomeric mouse alpha 2-macroglobulin: Characterization of the exons coding for the bait region. *Genomics* 22:530–539, 1994
21. Iwasaki H, Suzuki Y, Sinohara H: Cloning and sequencing of cDNAs encoding plasma alpha-macroglobulin and murinoglobulin from guinea pig: Implications for molecular evolution of alpha-macroglobulin family. *J Biochem* 120:1167–1175, 1996
22. Horn F, Wegenka UM, Lutticken C, Yuan J, Roeb E, Boers W, Buschmann J, Heinrich PC: Regulation of alpha 2-macroglobulin gene expression by interleukin-6. *Ann NY Acad Sci* 737:308–323, 1994
23. Saad B, Frei K, Scholl FA, Fontana A, Maier P: Hepatocyte-derived interleukin-6 and tumor-necrosis factor alpha mediate the lipopolysaccharide-induced acute-phase response and nitric oxide release by cultured rat hepatocytes. *Eur J Biochem* 229:349–355, 1995
24. Li AH, Zwain IH, Pineau C, Cazzolla N, Saso L, Silvestrini B, Bardin CW, Cheng CY: Response of alpha 2-macroglobulin messenger ribonucleic acid expression to acute inflammation in the testis is different from the response in the liver and brain. *Biol Reproduct* 50:1287–1296, 1994
25. Wegenka UM, Buschmann J, Lutticken C, Heinrich PC, Horn F: Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is rapidly activated by interleukin-6at the post-translational level. *Molec Cell Biol* 13:276–288, 1993
26. Brechner T, Hocke G, Goel A, Fey GH: Interleukin 6 response factor binds co-operatively at two adjacent sites in the promoter upstream region of the rat alpha 2-macroglobulin gene. *Molec Biol Med* 8:267–285, 1991
27. Fey GH, Gauldie J: The acute phase response of the liver in inflammation. *Progr Liver Dis* 9:89–116, 1990
28. Abraham LJ, Bradshaw AD, Fletcher RG, Fey GH: Interleukin 6 is a negative regulator of the acute phase alpha 1-inhibitor III gene. *Molec Biol Med* 7:261–271, 1990
29. Abraham LJ, Bradshaw AD, Shiels BR, Northemann W, Hudson G, Fey GH: Hepatic transcription of the acute-phase alpha 1-inhibitor III gene is controlled by a novel combination of cis-acting regulatory elements. *Molec Cell Biol* 10:3483–3491, 1990
30. Webb DJ, Wen J, Lysiak JJ, Umans L, Van Leuven F, Gonias SL: Murine alpha-macroglobulins demonstrate divergent activities as neutralizers of transforming growth factor-beta and as inducers of nitric oxide synthesis. A possible mechanism for the endotoxin insensitivity of the alpha2-macroglobulin gene knock-out mouse. *J Biol Chem* 271:24982–24988, 1996
31. Kurdowska A, Carr FK, Stevens MD, Baughman RP, Martin TR: Studies on the interaction of IL-8 with human plasma alpha 2-macroglobulin: Evidence for the presence of IL-8 complexed to alpha 2-macroglobulin in lung fluids of patients with adult respiratory distress syndrome. *J Immunol* 158:1930–1940, 1997
32. Lysiak JJ, Hussaini IM, Webb DJ, Glass WF II, Allietta M, Gonias SL: Alpha 2-macroglobulin functions as a cytokine carrier to induce nitric oxide synthesis and cause nitric oxide-dependent cytotoxicity in the RAW 264.7 macrophage cell line. *J Biol Chem* 270: 21919–21927, 1995
33. da Silva GC, Teixeira N, Bell SC: Major secretory product of the mesometrial decidua in the rat, a variant of alpha-2-macroglobulin, binds insulin-like growth factor I via a protease-dependent mechanism. *Molec Reproduct Develop* 44:103–110, 1996
34. Stefansson S, Lawrence DA, Argraves WS: Plasminogen activator inhibitor-1 and vitronectin promote the cellular clearance of thrombin

by low density lipoprotein receptor-related proteins 1 and 2. *J Biol Chem* 271:8215–8220, 1996
35. Valnickova Z, Thogersen IB, Christensen S, Chu CT, Pizzo SV, Enghild JJ: Activated human plasma carboxypeptidase B is retained in the blood by binding to alpha2-macroglobulin and pregnancy zone protein. *J Biol Chem* 271:12937–12943, 1996
36. Vaziri ND, Gonzales EC, Shayestehfar B, Barton CH: Plasma levels and urinary excretion of fibrinolytic and protease inhibitory proteins in nephrotic syndrome. *J Lab Clin Med* 124:118–124, 1994
37. Asami T, Nishihara T, Tomisawa S, Sakai K, Uchiyama M: Study on proteinase-inhibiting capacity of plasma alpha 2-macroglobulin in idiopathic nephrotic syndrome. *Nephron* 72:512–517, 1996
38. Karkar AM, Rees AJ: Influence of an established acute phase response on the severity of experimental nephritis. *Clin Exp Immunol* 98:295–299, 1994
39. Sambrook J, Fritsch EF, Maniatis T: *Molecular Cloning, A Laboratory Manual* (2nd ed). Cold Spring Harbor, Cold Spring Harbor Laboratory Press, 1989
40. Bertani T, Poggi A, Pozzoni R, Delaini F, Sacchi G, Thoua Y, Mecca G, Remuzzi G, Donati B: Adriamycin-induced nephrotic syndrome in rats: Sequence of pathologic event. *Lab Invest* 46:16–23, 1982
41. Orlando RA, Kerjaschki D, Kurihara H, Biemesderfer D, Farquhar MG: gp330 associates with a 44-kDa protein in the rat kidney to form the Heymann nephritis antigenic complex. *PNAS USA* 89:6698–6702, 1992
42. Kaysen GA, Watson JB: The mechanism of hypoalbuminemia in the 7/8 nephrectomized rat with chronic renal failure. *Am J Physiol* 243:F372–F378, 1982
43. Kaysen GA, Jones H Jr, Joles JA; van Tol A: Effect of plasma oncotic pressure on apolipoprotein levels in rats with Heymann nephritis. *Miner Electrolyte Metab* 22:31–38, 1996
44. Srivastava RA, Srivastava N, Schonfeld G: Expression of low density lipoprotein receptor, apolipoprotein AI, AII and AIV in various rat organs utilizing an efficient and rapid method for RNA isolation. *Biochem Int* 27:85–95, 1992
45. Davidsen O, Christensen EI, Gliemann J: The plasma clearance of human α2-macroglobulin-trypsin complex in the rat is mainly accounted for by uptake into hepatocytes. *Biochim Biophys Acta* 846: 85–92, 1985
46. Kaysen GA, Schoenfeld PY: Albumin homeostasis in patients undergoing continuous ambulatory peritoneal dialysis. *Kidney Int* 25:107–114, 1984
47. Katz J, Bonorris G, Sellers AL: Albumin metabolism in aminonucleoside nephrotic rats. *J Lab Clin Med* 62:910–934, 1963
48. Jensen H, Rossing N, Anderson SB, Jarnum S: Albumin metabolism in the nephrotic syndrome in adults. *Clin Sci* 33:445–457, 1967
49. Esumi H, Sato S, Okui M, Sugimura T, Nagase S: Turnover of serum proteins in rats with analbuminemia. *Biochem Biophys Res Comm* 87:1191–1199, 1979
50. Marsh JB, Sparks CE: Hepatic secretion of lipoproteins in the rat and the effect of experimental nephrosis. *J Clin Invest* 64:1229–1237, 1979
51. Marsh JB: Lipoprotein metabolism in experimental nephrosis. *J Lipid Res* 25:1619–1623, 1984
52. Cavus I, Koo PH, Teyler TJ: Inhibition of long-term potentiation development in rat hippocampal slice by alpha 2-macroglobulin, an acute-phase protein in the brain. *J Neurosci Res* 43:282–288, 1996
53. Abraham LJ, Bradshaw AD, Northemann W, Fey GH: Identification of a glucocorticoid response element contributing to the constitutive expression of the rat liver alpha 1-inhibitor III gene. *J Biol Chem* 266:18268–18275, 1991
54. Kirsch R, Frith L, Black E, Hoffenberg R: Regulation of albumin synthesis and catabolism by alteration of dietary protein. *Nature* 217:578–579, 1968
55. Kaysen GA, Gambertoglio J, Jiminez I, Jones H, Hutchison FN: Effect of dietary protein intake on albumin homeostasis in nephrotic patients. *Kidney Int* 29:572–577, 1986
56. Kaysen GA, Rathore V: Derangements of protein metabolism in chronic renal failure. *Blood Purif* 14:373–381, 1996
57. Sellers AL, Katz J, Bonorris G: Albumin distribution in the nephrotic rat. *J Lab Clin Med* 71:511–516, 1968
58. Katz J, Sellers AL, Bonorris G: Effect of nephrectomy on plasma albumin catabolism in experimental nephrosis. *J Lab Clin Med* 63:680–686, 1964
59. Galaske RG, Baldamus CA, Stolte H: Plasma protein handling in the rat kidney: Micropuncture experiments in the acute heterologous phase of anti-GBM-nephritis. *Pflügers Arch* 375:269–277, 1978
60. Eisenbach GM, Van Liew JB, Boylan JW: Effect of angiotensin on the filtration of protein in the rat kidney. A micropuncture study. *Kidney Int* 8:80–87, 1975
61. Landwehr DM, Carvalho JS, Oken DE: Micropuncture studies of the filtration and absorption of albumin by nephrotic rats. *Kidney Int* 11:9–17, 1977
62. Lewy JE, Pesce A: Micropuncture study of albumin transfer in aminonucleoside nephrosis in the rat. *Pediatr Res* 7:553–559, 1973
63. Oken DE, Cotes SC, Mende CW: Micropuncture study of tubular transport of albumin in rats with aminonucleoside nephrosis. *Kidney Int* 1:3–11, 1972
64. Katz J, Sellers AL, Bonorris G: Plasma albumin metabolism during transient renin proteinuria. *J Lab Clin Med* 64:709–716, 1964